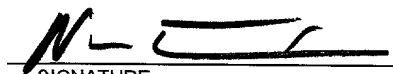


U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER <b>KE INAN=3</b>
<b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b>		U S APPLICATION NO (If known, see 37 CFR 1.5) <b>09/914268</b>
INTERNATIONAL APPLICATION NO. <b>PCT/IL99/00112</b>	INTERNATIONAL FILING DATE <b>24 February 1999</b>	PRIORITY CLAIMED
TITLE OF INVENTION <b>METHOD AND KIT FOR THE DETECTION OF EXPLOSIVES</b>		
APPLICANT(S) FOR DO/EO/US <b>Ehud KEINAN et al.</b>		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li>4. <input checked="" type="checkbox"/> The US has been elected in a Demand by the expiration of 19 months from the priority date (PCT Article 31).</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is attached hereto (required only if not transmitted by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).</li> <li>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> have been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired</li> <li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4))</li> <li>10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol> <p><b>Items 11. to 16. below concern document(s) or information included:</b></p> <ol style="list-style-type: none"> <li>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98</li> <li>12. <input type="checkbox"/> An Assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>13. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment.  <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li>14. <input type="checkbox"/> A substitute specification.</li> <li>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>16. <input checked="" type="checkbox"/> Other items or information:             <ul style="list-style-type: none"> <li><input checked="" type="checkbox"/> Courtesy copy of the International Application as filed.</li> <li><input checked="" type="checkbox"/> Courtesy copy of the first page of the International Publication (WO 99/43846).</li> <li><input checked="" type="checkbox"/> Courtesy copy of the International Preliminary Examination Report. There were no annexes.</li> <li><input checked="" type="checkbox"/> Courtesy Copy of the International Search Report.</li> </ul> </li> </ol>		

U.S. APPLICATION NO (If known, see 37 CFR 1.5)	International Application No <b>PCT/IL99/00112</b>	Attorney's Docket No <b>KE INAN=3</b>																																																				
<p>17. [xx] The following fees are submitted:</p> <p><b>BASIC NATIONAL FEE (37 CFR 1.492 (a)(1)-(5):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....<b>\$1000.00</b></p> <p>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....<b>\$860.00</b></p> <p>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....<b>\$710.00</b></p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4).....<b>\$690.00</b></p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....<b>\$100.00</b></p> <p style="text-align: center;"><b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b></p> <p>Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than [ ] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).</p> <table border="1" style="width: 100%; border-collapse: collapse;"><thead><tr><th style="width: 25%;">Claims as Originally Presented</th><th style="width: 15%;">Number Filed</th><th style="width: 15%;">Number Extra</th><th style="width: 15%;">Rate</th><th style="width: 10%;"></th><th style="width: 20%;"></th></tr></thead><tbody><tr><td>Total Claims</td><td>35 - 20</td><td>15</td><td>X \$18.00</td><td>\$</td><td></td></tr><tr><td>Independent Claims</td><td>2 - 3</td><td></td><td>X \$80.00</td><td>\$</td><td></td></tr><tr><td>Multiple Dependent Claims (if applicable)</td><td></td><td></td><td>+\$270.00</td><td>\$</td><td></td></tr></tbody></table> <p style="text-align: center;"><b>TOTAL OF ABOVE CALCULATIONS =</b></p> <table border="1" style="width: 100%; border-collapse: collapse;"><thead><tr><th style="width: 25%;">Claims After Post Filing Prel. Amend</th><th style="width: 15%;">Number Filed</th><th style="width: 15%;">Number Extra</th><th style="width: 15%;">Rate</th><th style="width: 10%;"></th><th style="width: 20%;"></th></tr></thead><tbody><tr><td>Total Claims</td><td>- 20</td><td></td><td>X \$18.00</td><td>\$</td><td></td></tr><tr><td>Independent Claims</td><td>- 3</td><td></td><td>X \$78.00</td><td>\$</td><td></td></tr></tbody></table> <p style="text-align: center;"><b>TOTAL OF ABOVE CALCULATIONS =</b></p> <p>Reduction of 1/2 for filing by small entity, if applicable. Applicant claims small entity status. See 37 CFR 1.27.</p> <p style="text-align: center;"><b>SUBTOTAL =</b></p> <p>Processing fee of <b>\$130.00</b> for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).</p> <p style="text-align: center;"><b>TOTAL NATIONAL FEE =</b></p> <p>Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property +</p> <p style="text-align: center;"><b>TOTAL FEES ENCLOSED =</b></p> <table border="1" style="width: 100%; border-collapse: collapse;"><tr><td style="width: 80%;"></td><td style="width: 20%; text-align: center;"><b>Amount to be:</b></td><td style="width: 10%;"></td></tr><tr><td></td><td style="text-align: center;"><b>refunded</b></td><td style="text-align: center;">\$</td></tr><tr><td></td><td style="text-align: center;"><b>charged</b></td><td style="text-align: center;">\$</td></tr></table>		Claims as Originally Presented	Number Filed	Number Extra	Rate			Total Claims	35 - 20	15	X \$18.00	\$		Independent Claims	2 - 3		X \$80.00	\$		Multiple Dependent Claims (if applicable)			+\$270.00	\$		Claims After Post Filing Prel. Amend	Number Filed	Number Extra	Rate			Total Claims	- 20		X \$18.00	\$		Independent Claims	- 3		X \$78.00	\$			<b>Amount to be:</b>			<b>refunded</b>	\$		<b>charged</b>	\$	<p style="text-align: center;"><b>CALCULATIONS PTO USE ONLY</b></p>	
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<p>a. [ ] A check in the amount of \$ _____ to cover the above fees is enclosed.</p> <p>b. [X] Credit Card Payment Form (PTO-2038), authorizing payment in the amount of \$ 430.00, is attached.</p> <p>c. [ ] Please charge my Deposit Account No. <b>02-4035</b> in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.</p> <p>d. [ ] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <b>02-4035</b>. A duplicate copy of this sheet is enclosed.</p> <p><b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b></p> <p>SEND ALL CORRESPONDENCE TO:</p> <p><b>BROWDY AND NEIMARK, P.L.L.C.</b> <b>624 NINTH STREET, N.W., SUITE 300</b> <b>WASHINGTON, D.C. 20001</b> <b>TEL: (202) 628-5197</b> <b>FAX: (202) 737-3528</b> Date of this submission: <b>August 24, 2001</b></p>			<p style="text-align: center;"> SIGNATURE <u>Norman J. Latker</u> NAME <u>19,963</u> REGISTRATION NUMBER</p>																																																			

Rec'd PCT/PTO 15 FEB 2002

#5/  
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Art Unit:
Ehud KEINAN	)	
IA No.: PCT/IL99/00112	)	
IA Filed: February 24, 1999	)	Washington, D.C.
U.S. App. No.: 09/914,268	)	
National Filing Date:	)	February 15, 2002
August 24, 2001	)	
For: METHOD AND KIT FOR THE	)	Docket No.: KEINAN=3
DETECTION OF EXPLOSIVES	)	

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents and Trademarks  
Washington, D.C. 20231

Sir:

Prior to examination upon the merits, kindly amend  
as follows:

IN THE CLAIMS

3. (Amended) A method according to Claim 1 or 2, wherein the solvent is selected from the group consisting of lower alkanols, dimethylsulfoxide, N, N-dimethylformamide, carboxylic acids, especially acetic acid and trifluoroacetic acid, and sulfonic acids.

5. (Amended) A method according to Claim 1, wherein the strong acid is selected from the group consisting of H<sub>2</sub>SO<sub>4</sub>, HCl, HBr, HClO<sub>4</sub>, H<sub>3</sub>PO<sub>2</sub>, H<sub>3</sub>PO<sub>3</sub>, H<sub>3</sub>PO<sub>4</sub> and HNO<sub>3</sub>.

8. (Amended) A method according to Claim 1, wherein the pH is adjusted by said buffer to about 5.0 to about 9.0.

9. (Amended) A method according to Claim 8, wherein the buffer is about 0.01 to 0.5 M citrate/phosphate buffer.

10. (Amended) A method according to Claim 1, wherein the peroxidase enzyme is Horseradish peroxidase.

11. (Amended) A method according to Claim 2, wherein the substrate is selected from the group consisting of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt, 2,7-diaminofluorene, 3,3',5,5'-tetramethylbenzidine and its dihydrochloride salt, 5-aminosalicylic acid, o-phenylenediamine and its dihydrochloride salt, 5-amino-2,3-dihydro-1,4-phthalazinedione, 3-amino-9-ethylcarbazole, 4-chloro-1-naphthol, 3,3'-diaminobenzidine, o-dianisidine and its dihydrochloride salt, guaiacol and pyrogallol.

12. (Amended) A method according to Claim 1, wherein the sample is introduced into a mixture of the organic solvent and the aqueous solution of the strong acid.

13. (Amended) A method according to Claim 1, wherein the peroxidase enzyme is combined with the buffer prior to being contacted with said resulting mixture.

14. (Amended) A method according to Claim 1, wherein the buffer is combined with the substrate prior to being contacted with said resulting mixture.

15. (Amended) A method according to Claim 1, wherein said resulting mixture is contacted with a combination of the buffer, the peroxidase enzyme and the substrate.

19. (Amended) A method according to Claim 16, wherein the peroxidase enzyme is Horseradish peroxidase.

21. (Amended) A kit for use in the method of Claim 3, comprising a packaged mixture of organic solvent and aqueous solution of a strong acid, packaged buffer, packaged peroxidase enzyme and packaged substrate.

22. (Amended) A kit according to Claim 20, wherein the buffer and the peroxidase enzyme are packaged together.

23. (Amended) A kit according to Claim 20, wherein the buffer and the substrate are packaged together.

24. (Amended) A kit according to Claim 20, wherein the buffer, the peroxidase enzyme and the substrate are packaged together.

25. (Amended) A kit according to Claim 20, comprising a plurality of sealed ampoules each containing the buffer and the peroxidase enzyme.

In re of: KEINAN=3

28. (Amended) A kit according to Claim 20, wherein the organic solvent is acetic acid.

29. (Amended) A kit according to Claim 20, wherein the strong acid is aqueous sulfuric acid.

30. (Amended) A kit according to Claim 20, wherein the peroxidase enzyme is Horseradish peroxidase.

31. (Amended) A kit according to Claim 20, wherein the buffer is citrate/phosphate buffer.

32. (Amended) A kit according to Claim 20, further comprising a plurality of small open receptacles for carrying out the test therein.

If, inadvertently, a proper multiple dependent claim has not been amended to reduce it to single dependency, please amend it to be dependent solely on the first-mentioned claim, or, if that is not possible, please cancel the claim and notify the undersigned.

#### REMARKS

The above amendments to the claims are being made in order to eliminate multiple dependency and for the purpose of reducing the filing fee. Please enter this amendment prior to calculation of the filing fee in this case.

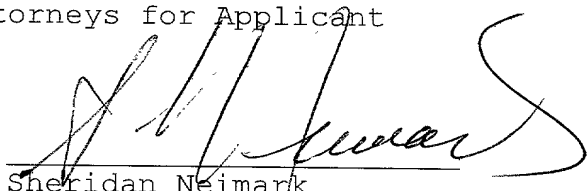
Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with Markings to Show Changes Made."

Favorable consideration and allowance are earnestly solicited.

In re of: KEINAN=3

Respectfully submitted,  
BROWDY AND NEIMARK, P.L.L.C.  
Attorneys for Applicant

By:

  
Sheridan Neimark

Registration No. 20,520

SN:sfg

Telephone No.: (202) 628-5197

Facsimile No.: (202) 737-3528

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

3. A method according to Claim 1 or 2, wherein the solvent is selected from the group consisting of lower alkanols, dimethylsulfoxide, N, N-dimethylformamide, carboxylic acids, especially acetic acid and trifluoroacetic acid, and sulfonic acids.
5. A method according to Claim 1 [any one of Claims 1 to 4], wherein the strong acid is selected from the group consisting of H<sub>2</sub>SO<sub>4</sub>, HCl, HBr, HClO<sub>4</sub>, H<sub>3</sub>PO<sub>2</sub>, H<sub>3</sub>PO<sub>3</sub>, H<sub>3</sub>PO<sub>4</sub> and HNO<sub>3</sub>.
8. A method according to Claim 1 [any of Claims 1 to 7], wherein the pH is adjusted by said buffer to about 5.0 to about 9.0.
9. A method according to Claim 8 [any of Claims 1 to 8], wherein the buffer is about 0.01 to 0.5 M citrate/phosphate buffer.
10. A method according to Claim 1 [any of Claims 1 to 9], wherein the peroxidase enzyme is Horseradish peroxidase.
11. A method according to Claim 2 [any one of claims 2 to 10], wherein the substrate is selected from the group consisting of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt, 2, 7-diaminofluorene, 3,3',5,5'-tetramethylbenzidine and its dihydrochloride salt, 5-aminosalicylic acid, o-phenylenediamine and its dihydrochloride salt, 5-amino-2,3-dihydro-1,4-phthalazinedione, 3-amino-9-ethylcarbazole, 4-chloro-1-naphthol, 3,3'-diaminobenzidine, o-dianisidine and its dihydrochloride salt, guaiacol and pyrogallol.
12. A method according to Claim 1 [any one of Claims 1 to 11], wherein the sample is introduced into a mixture of the organic solvent and the aqueous solution of the strong acid.
13. A method according to Claim 1 [any one of the Claims 1 to 12], wherein the peroxidase enzyme is combined with the buffer prior to being contacted with said resulting mixture.
14. A method according to Claim 1 [any one of Claims 1 to 12], wherein the buffer is combined with the substrate prior to being contacted with said resulting mixture.
15. A method according to Claim 1 [any one of Claims 1 to 12], wherein said resulting mixture is contacted with a

combination of the buffer, the peroxidase enzyme and the substrate.

19. A method according to Claim 16 [any one of Claims 16 to 18], wherein the peroxidase enzyme is Horseradish peroxidase.

21. A kit for use in the method of Claim 3 [Claims 3 or 13], comprising a packaged mixture of organic solvent and aqueous solution of a strong acid, packaged buffer, packaged peroxidase enzyme and packaged substrate.

22. A kit according to Claim 20 [Claim 20 or 21], wherein the buffer and the peroxidase enzyme are packaged together.

23. A kit according to Claim 20 [Claims 20 or 21], wherein the buffer and the substrate are packaged together.

24. A kit according to Claim 20 [Claims 20 or 21], wherein the buffer, the peroxidase enzyme and the substrate are packaged together.

25. A kit according to Claim 20 [Claims 20 or 21], comprising a plurality of sealed ampoules each containing the buffer and the peroxidase enzyme.

28. A kit according to Claim 20 [anyone of Claims 20 to 27], wherein the organic solvent is acetic acid.

29. A kit according to Claim 20 [any one of Claims 20 to 28], wherein the strong acid is aqueous sulfuric acid.

30. A kit according to Claim 20 [any one of Claims 20-28], wherein the peroxidase enzyme is Horseradish peroxidase.

31. A kit according to Claim 20 [any one of Claims 20 to 30], wherein the buffer is citrate/phosphate buffer.

32. A kit according to Claim 20 [any one of Claims 20 to 31], further comprising a plurality of small open receptacles for carrying out the test therein.



#4/a

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Art Unit:
Ehud KEINAN et al.	)	
IA No.: PCT/IL99/00112	)	
IA Filed: February 24, 1999	)	Washington, D.C.
U.S. App. No.:	)	
(Not Yet Assigned)	)	
National Filing Date:	)	August 24, 2001
(Not Yet Received)	)	
For: METHOD AND KIT FOR...	)	Docket No.: KEINAN=3

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents and Trademarks  
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior to calculation of the filing fee, kindly amend as follows:

IN THE SPECIFICATION

After the title please insert the following paragraph:

REFERENCE TO RELATED APPLICATIONS

The present application is the national stage under 35 U.S.C. 371 of international application PCT/IL99/00112, filed February 24, 1999 which designated the United States, and which international application was published under PCT Article 21(2) in the English language.

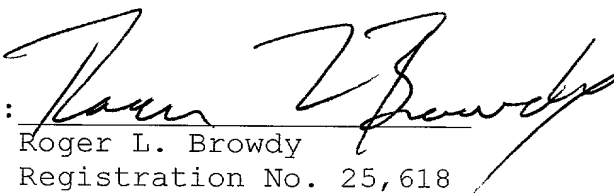
In re of: Ehud KEINAN et al. (KEINAN=3)

REMARKS

The above amendment to the specification is being made to insert reference to the PCT application of which the present case is a U.S. national stage.

Favorable consideration is earnestly solicited.

Respectfully submitted,  
BROWDY AND NEIMARK, P.L.L.C.  
Attorneys for Applicant

By:   
Roger L. Browdy  
Registration No. 25,618

RLB:wrđ

Telephone No.: (202) 628-5197

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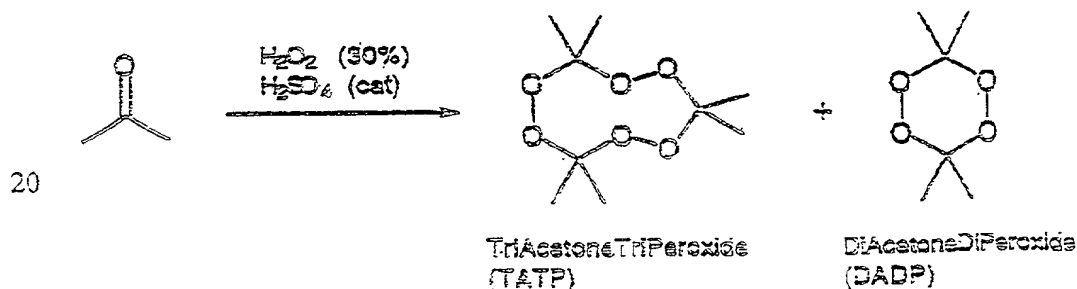
518 Rec'd PCT/PTO 24 AUG 2001

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METHOD AND KIT FOR THE DETECTION OF EXPLOSIVES**FIELD OF THE INVENTION**

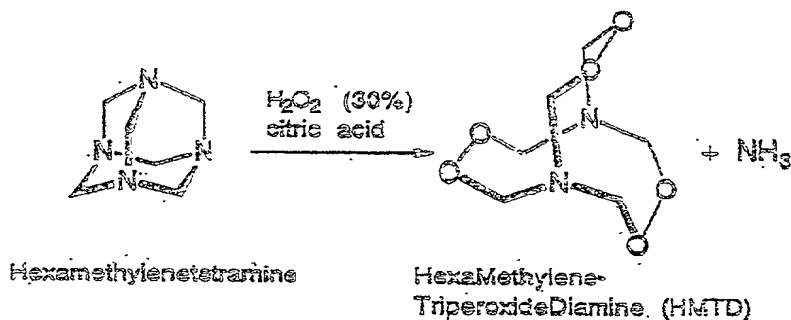
This invention concerns a method of detecting peroxide-based explosives and a kit for use in this method.

Improvised explosive devices based on peroxide containing materials have increasingly been used in recent years by various terrorist organizations, especially in Israel, as well as in the UK and the USA. The main reason is that such peroxide-based explosives can be easily "home-made" using inexpensive, readily available starting materials which can be purchased in most hardware and paint stores, even in bulk quantities. One class of such peroxide-based explosives can be easily produced by reacting various carbonyl compounds (e.g. ketones, aldehydes and their derivatives) with hydrogen peroxide under acid catalysis. For example, when a mixture of acetone, hydrogen peroxide and small amounts of a mineral acid, e.g. sulfuric acid, is left for several hours at room temperature, white crystals of triacetone triperoxide (hereinafter "TATP") and diacetone diperoxide (hereinafter "DADP") are formed by the following reaction :



These crystals are collected and can be washed with water or with 10% sodium carbonate solution. TATP and DADP are powerful initiators by themselves and can be used as the main filler in home-made detonators. They are quite unstable explosives and may explode under rough handling, scratching with metals or by sparks and open flame, even when they contain up to 25% water or even when immersed in water. The explosive intensity of TATP is approximately 5/8 that of TNT. This material is quite volatile, unless used shortly after its manufacture, should be stored in a cool, dark dry place. It has been reported that at room temperature TATP loses 2/3 of its weight within 14 days and at 50° C it evaporates completely within 40 days.

Another commonly used peroxide based explosive is hexamethylenetriperoxidediamine (hereinafter "HMTD"). It can be conveniently prepared by treating hexamethylenetetramine with hydrogen peroxide in the presence of a weak acid, such as citric acid, in order to neutralize the liberated ammonia. The reaction can be represented as follows:



HMTD is almost insoluble in water and in common organic solvents at room temperature. It is too active and too unstable to be of commercial use as an explosive.

Although many peroxide containing materials of the above-described type are known for more than 70 years, no satisfactory method for their detection has been suggested to date. The detection of peroxide-based explosives is particularly difficult because all these materials do not contain

nitro groups or any other nitrogen oxide functional groups. Since most of the currently available explosive detectors are based on the detection of nitro groups, they cannot be employed for detection of peroxide-based materials. Consequently, and in view of the increased use of such peroxide-based explosives by terrorists, especially in the Middle East as well as in other parts of the world, there exists an urgent need for highly sensitive methods and devices for the early detection of peroxide-based explosives and improvised explosive devices employing them.

## 10 OBJECT OF THE INVENTION

It is thus the object of the present invention to provide a reliable method for the fast and easy detection of peroxide-based explosives.

It is a further object of the invention to provide a portable kit for the simple yet reliable and selective detection and identification of peroxide based explosives.

## SUMMARY OF THE INVENTION

The above object was achieved by the present invention which provides a method of detecting a peroxide-based explosive in a sample suspected of consisting of or comprising such explosive, which method comprises dissolving said sample in a suitable organic solvent, contacting the solution with an aqueous solution of a strong acid capable of decomposing said explosive to release hydrogen peroxide, and contacting the resulting mixture with a peroxidase enzyme, a buffer to adjust the pH to such permitting action of the peroxidase enzyme and a substrate capable of being oxidized by the oxidant under the catalysis of the peroxidase enzyme to produce a pronounced change in a measurable physical parameter of the substrate.

The invention also provides, in a second aspect thereof, a kit for use in the method of the invention, comprising packaged organic solvent,

packaged aqueous solution of a strong acid, packaged buffer, packaged peroxidase enzyme and packaged substrate.

#### DETAILED DESCRIPTION OF THE INVENTION

5 In accordance with the method of the present invention, a sample to be tested for the presence of peroxide-based explosive, is dissolved in a suitable organic solvent and contacted with a strong acid in the presence of water, whereby any peroxide-containing material is decomposed to release free hydrogen peroxide. The resulting mixture is neutralized with a suitable buffer  
10 and the presence of peroxide in the mixture is detected by means of a peroxidase enzyme and a substrate which is oxidized with a resulting pronounced change in a measurable physical parameter thereof. When a such a change is observed the result of the test is a positive indication of the presence of peroxide-based explosive in the tested sample.

15 The peroxide enzyme serves as a catalyst in the above process, so that only a small amount of the enzyme is needed for the oxidation of comparatively large amounts of the substrate. Any readily available peroxidase enzyme can be used, for example Horseradish peroxidase or soy bean peroxidase. A preferred peroxidase enzyme is the Horseradish peroxidase  
20 which is highly selective for hydrogen peroxide.

The invention also contemplates the use of a peroxidase enzyme which is immobilized on a solid support, for example on a probe which can be introduced into the test mixture or on the inner surface of a small receptacle wherein the test mixture can be introduced.

25 The term "substrate" is used, within the context of the present invention, to refer to a compound capable of being oxidized by hydrogen peroxide in the presence of a peroxidase enzyme to yield a product exhibiting a pronounced change in at least one measurable physical parameter as compared to the unoxidized compound. In accordance with a preferred embodiment of

the invention this measurable physical parameter is the colour or colour intensity.

Examples of organic solvents suitable for use in the method of the present invention are, for example, tetrahydrofuran, 1,4-dioxane, lower  
5 alkanols, dimethylsulfoxide, N,N-dimethylformamide, carboxylic acids and sulfonic acids, especially acetic acid and trifluoroacetic acid. Preferred solvents are tetrahydrofuran, 1,4-dioxane and acetic acid.

Strong acids which can be used in the method of the present invention are, for example  $H_2SO_4$ , HCl, HBr,  $HClO_4$ ,  $H_3PO_2$ ,  $H_3PO_3$ ,  $H_3PO_4$   
10 and  $HNO_3$ . A preferred acid is sulfuric acid which can be used in concentrations of from about 5% to 95%, preferably from about 10% to about 50% by volume in water.

In accordance with an embodiment of the invention the sample to be tested can be introduced into a mixture of the organic solvent and the  
15 aqueous solution of the strong acid.

In order to enable the enzymatic reaction of the peroxidase enzyme with the hydrogen peroxide and the substrate, it is necessary to neutralize the acidic mixture of the organic solvent and the aqueous strong acid containing the sample to be tested, so as to adjust its pH to a value between  
20 about 5.0 to about 9.0. This can be achieved by a suitable buffer system which should be added to the mixture before the addition of the peroxidase enzyme or simultaneously therewith. In accordance with one embodiment of the invention the buffer is added to the mixture together with the substrate before the mixture is contacted with the peroxidase enzyme. Yet another preferred procedure  
25 comprises adding the buffer, the substrate and the peroxidase enzyme simultaneously.

As suitable buffer systems for use in the method of the invention there may be mentioned citrate/phosphate buffer, acetate buffer, phthalate buffer, citrate buffer, phosphate buffer, imidazole buffer, triethanolamine

buffer, tris(hydroxymethyl)aminomethane buffer, bis-tris buffer and bis-tris propane buffer. A preferred buffer system is citrate/phosphate buffer 0.1M at pH 5.0.

Thus, in accordance with a preferred embodiment the invention  
5 provides a method of detecting a peroxide-based explosive in a sample suspected of consisting of or comprising such explosive, which method comprises introducing said sample into a mixture of an organic solvent and an aqueous solution of a strong acid capable of decomposing said explosive to release hydrogen peroxide, and contacting the resulting mixture with a solution  
10 comprising a peroxidase enzyme, a buffer to adjust the pH to such permitting action of the peroxidase enzyme and a substrate capable of being oxidized by oxidant under the catalysis of the peroxidase enzyme to produce a pronounced change in the colour of the substrate or its colour intensity.

In this embodiment, the organic solvent is preferably acetic acid  
15 and the strong acid is preferably aqueous sulfuric acid 50% by volume. The preferred peroxidase enzyme is Horseradish peroxidase.

In accordance with a further aspect thereof, the invention provides a kit for the detection of a peroxide-based explosive in a sample suspected of consisting of or comprising such explosive, which kit comprises  
20 packaged organic solvent, packaged aqueous solution of a strong acid, packaged buffer solution, packaged peroxidase enzyme and packaged substrate.

In a preferred kit according to the invention a mixture of the organic solvent and the aqueous solution of the strong acid are packaged together in the same container. In accordance with another modification of the  
25 kit according to the invention the buffer and the substrate are packaged together in the same container.

In accordance with a more preferred embodiment of the kit according to the invention the buffer, the peroxidase enzyme and the substrate are all packaged together. Conveniently the kit according to the invention



comprises a plurality of sealed ampoules each containing the peroxidase enzyme, optionally in admixture with the buffer and the substrate, in an amount sufficient for carrying out one test.

The kit according to the present invention may also include a plurality of small reaction vessels, for example small dishes or open receptacles, for carrying out the test therein.

The invention will now be described in more detail in the following non-limit examples.

10      Example 1

A few crystals of TATP (about 1-2mg) were placed in a shallow well, 0.1 ml of 1,4-dioxane 0.1ml was added and than 0.1ml of sulfuric acid 50% v/v in water. The reaction mixture was allowed to stand for 30 seconds before the substrate 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt in 0.2ml of citrate/ phosphate buffer (0.1M at pH 5.0) was added, followed by 0.05ml of Horseradish peroxidase 5 mg/ml in citrate/phosphate buffer (0.1M at pH 5.0 ml.). An intense bluish-green colour developed in less than 30 seconds.

20

Example 2

The procedure of Example 1 was repeated using 1,4-dioxane as solvent instead of the tetrahydrofuran. The same result was obtained.

25

Example 3

The procedure of Example 1 was repeated using 2,7-diaminofluorene as the substrate. A blue-green colour developed in less than 30 seconds.

Example 4

The procedure of Example 1 was repeated using 3,3',5,5'-tetramethylbenzidine dihydrochloride as the substrate.

5 An immediate blue colour formation was observed.

Example 5

The procedure of Example 1 was repeated using 5-aminosalicylic acid as the substrate. A brown colour developed rapidly.

10

Example 6

The procedure of Example 1 was repeated using o-phenylenediamine dihydrochloride as the substrate. An orange colour developed in less than 30 seconds.

15

Example 7

A few crystals of TATP (about 1-2mg) were placed in a shallow well, 0.1ml of a 1:1 mixture of sulfuric acid 50% v/v in water and acetic acid was added and the reaction mixture was allowed to stand for 30 seconds before 0.2ml of a 4:1 mixture of the substrate 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt and the enzyme Horseradish peroxidase 5 mg/ml in citrate/phosphate buffer (0.1 M at pH 5.0) was added. An intense bluish-green colour developed in less than 30 seconds.

20

25

Example 8

The procedure of Example (7) was repeated using the substrates of Examples 3-6. The same results as in these examples were observed.

## CLAIMS:

1. A method of detecting a peroxide-based explosive in a sample suspected of consisting of or comprising such explosive, which method comprises  
5 dissolving said sample in a suitable organic solvent, contacting the solution with an aqueous solution of a strong acid capable of decomposing said explosive to release hydrogen peroxide, and contacting the resulting mixture with a peroxidase enzyme, a buffer to adjust the pH to such permitting action of the peroxidase enzyme and a substrate capable of being oxidized by the oxidant  
10 under the catalysis of the peroxidase enzyme to produce a pronounced change in a measurable physical parameter of the substrate.
2. A method according to Claim 1, wherein said physical parameter of the substrate is its colour or colour intensity  
15
3. A method according to Claim 1 or 2, wherein the solvent is selected from tetrahydrofuran, 1,4-dioxane, lower alkanols, dimethylsulfoxide, N,N-dimethylformamide, carboxylic acids, especially acetic acid and trifluoroacetic acid, and sulfonic acids.  
20
4. A method according to Claim 3, wherein the organic solvent is acetic acid.
5. A method according to any one of Claims 1 to 4, wherein the strong acid  
25 is selected from  $\text{H}_2\text{SO}_4$ ,  $\text{HCl}$ ,  $\text{HBr}$ ,  $\text{HClO}_4$ ,  $\text{H}_3\text{PO}_2$ ,  $\text{H}_3\text{PO}_3$ ,  $\text{H}_3\text{PO}_4$  and  $\text{HNO}_3$ .
6. A method according to Claim 5, wherein the strong acid is  $\text{H}_2\text{SO}_4$ .

7. A method according to Claim 6, wherein the concentration of the aqueous  $H_2SO_4$  solution is from about 5% to 95%, preferably from about 10% to about 50% by volume.
- 5 8. A method according to any one of Claims 1 to 7, wherein the pH is adjusted by said buffer to about 5.0 to about 9.0.
9. A method according to any one of Claims 1 to 8, wherein the buffer is about 0.01 to 0.5 M citrate/phosphate buffer.
- 10 10. A method according to any one of Claims 1 to 9, wherein the peroxidase enzyme is Horseradish peroxidase.
11. A method according to any one of Claims 2 to 10, wherein the substrate  
15 is selected from 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt, 2,7-diaminofluorene, 3,3',5,5'-tetramethylbenzidine and its dihydrochloride salt, 5-aminosalicylic acid, o-phenylenediamine and its dihydrochloride salt, 5-amino-2,3-dihydro-1,4-phthalazinedione, 3-amino-9-ethylcarbazole, 4-chloro-1-naphthol, 3,3'-diaminobenzidine, o-dianisidine and  
20 its dihydrochloride salt, guaiacol and pyrogallol.
12. A method according to any one of Claims 1 to 11, wherein the sample is introduced into a mixture of the organic solvent and the aqueous solution of the strong acid.
- 25 13. A method according to any one of Claims 1 to 12, wherein the peroxidase enzyme is combined with the buffer prior to being contacted with said resulting mixture.

14. A method according to any one of Claims 1 to 12, wherein the buffer is combined with the substrate prior to being contacted with said resulting mixture.

5 15. A method according to any one of Claims 1 to 12, wherein said resulting mixture is contacted with a combination of the buffer, the peroxidase enzyme and the substrate.

10 16. A method of detecting a peroxide-based explosive in a sample suspected of consisting of or comprising such explosive, which method comprises introducing said sample into a mixture of an organic solvent and an aqueous solution of a strong acid capable of decomposing said explosive to release hydrogen peroxide, and contacting the resulting mixture with a solution comprising a peroxidase enzyme, a buffer to adjust the pH to such permitting  
15 action of the peroxidase enzyme and a substrate capable of being oxidized by the oxidant under the catalysis of the peroxidase enzyme to produce a pronounced change in the colour of the substrate or its colour intensity.

20 17. A method according to Claim 16, wherein the organic solvent is acetic acid.

18. A method according to Claims 16 or 17, wherein the strong acid is aqueous sulfuric acid.

25 19. A method according to any one of Claims 16 to 18, wherein the peroxidase enzyme is Horseradish peroxidase.

20. A kit for use in the method of Claim 1, comprising packaged organic solvent, packaged aqueous solution of a strong acid, packaged buffer, packaged peroxidase enzyme and packaged substrate.
- 5 21. A kit for use in the method of Claims 3 or 13, comprising a packaged mixture of organic solvent and aqueous solution of a strong acid, packaged buffer, packaged peroxidase enzyme and packaged substrate.
- 10 22. A kit according to Claims 20 or 21, wherein the buffer and the peroxidase enzyme are packaged together.
23. A kit according to Claims 20 or 21, wherein the buffer and the substrate are packaged together.
- 15 24. A kit according to Claims 20 or 21, wherein the buffer, the peroxidase enzyme and the substrate are packaged together.
25. A kit according to Claims 20 or 21, comprising a plurality of sealed ampoules each containing the peroxidase enzyme.
- 20 26. A kit according to Claim 22, comprising a plurality of sealed ampoules each containing the buffer and the peroxidase enzyme.
27. A kit according to Claim 24, comprising a plurality of sealed ampoules
- 25 each containing the buffer, the peroxidase enzyme and the substrate.
28. A kit according to any one of Claims 20 to 27, wherein the organic solvent is acetic acid.

29. A kit according to any one of Claims 20 to 28, wherein the strong acid is aqueous sulfuric acid.
30. A kit according to any one of Claims 20-28, wherein the peroxidase  
5 enzyme is Horseradish peroxidase.
31. A kit according to any one of Claims 20 to 30, wherein the buffer is citrate/phosphate buffer.
- 10 32. A kit according to any one of Claims 20 to 31, further comprising a plurality of small open receptacles for carrying out the test therein.



## Combined Declaration for Patent Application and Power of Attorney

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

the specification of which (check one)

- [ ] is attached hereto;  
 [ ] was filed in the United States under 35 U.S.C. §111 on \_\_\_\_\_, as  
 U.S. Appl. No. \_\_\_\_\_\*; or  
 [x] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international  
 (PCT) application, PCT/IL99/00112 filed 24 February, 1999, entry requested on \_\_\_\_\_\*;  
 national stage application received U.S. Appl. No. \_\_\_\_\_\*; §371/§102(e) date \_\_\_\_\_\*  
 (\* if known)

and was amended on \_\_\_\_\_ (if applicable).  
*(include dates of amendments under PCT Art. 19 and 34 if PCT)*

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119 (a)-(d) and 365 (b) of any prior foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or under §365(a) of any PCT application which designated at least one country other than the U.S., listed below:

Application No.	Country	Filing Date (MM/DD/YYYY)
<u>123451</u>	<u>ISRAEL</u>	<u>February 2, 1998</u>

If I claimed foreign priority above, I hereby identify below any foreign application for patent (including an international (PCT) application designating a country other than the United States) or for an inventor's or plant breeder's certificate, having a filing date before that of the earliest application from which foreign priority is claimed (if left blank, then there are none):

Non-Priority Application No.	Country	Filing Date (MM/DD/YYYY)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional applications listed below:

Application No.	Filing Date (MM/DD/YYYY)

I hereby claim the benefit under 35 U.S.C. §120 of any prior U.S. non-provisional application(s) or under §365(c) of any prior PCT international application(s) designating the U.S., listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT international application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Filing Date (MM/DD/YYYY)	Status (patented, pending, abandoned)

As a named inventor, I hereby appoint the following registered practitioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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Title: Method and Kit for the Detection of ExplosivesU.S. Application filed August 24, 2001, Serial No. \_\_\_\_\_PCT Application filed February 25, 1999, Serial No. PCT/IL99/00112

The undersigned hereby authorizes the U.S. Attorneys or Agents appointed herein to accept and follow instructions from \_\_\_\_\_ as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorneys or Agents and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents appointed herein will be so notified by the undersigned.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			
FULL NAME OF SIXTH JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			

ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING. ALL ALTERATIONS MUST BE INITIALED AND DATED BY ALL INVENTORS PRIOR TO EXECUTION. NO ALTERATIONS CAN BE MADE AFTER THE DECLARATION IS SIGNED. ALL PAGES OF DECLARATION MUST BE SIGNED BY ALL INVENTORS.